5

15

20

25

30

Claims

- An isolated Nod-factor binding element comprising one or more isolated NFR polypeptide having a specific Nod-factor binding property, or a functional fragment thereof, wherein the NFR amino acid sequence is at least 60% identical to any one of SEQ ID NO: 8, 15 or 25.
- The Nod-factor binding element of claim 1, wherein said NFR
 polypeptide is NFR1, comprising the amino acid sequence selected from the group consisting of SEQ ID No: 24, 25, 52 and 54.
 - 3. The Nod-factor binding element of claim 1, wherein the NFR polypeptide is NFR5 comprising an amino acid sequence selected from the group consisting of SEQ ID No: 8, 15, 32, 40 and 48.
 - 4. The Nod-factor binding element of claim 1, comprising a NFR1 polypeptide or a functional fragment thereof, having the amino acid sequence selected from the group consisting of SEQ ID No: 24, 25, 52 and 54, and a NFR5 polypeptide or a functional fragment thereof, having an amino acid sequence selected from the group consisting of SEQ ID No: 8, 15, 32, 40 and 48.
 - 5. An isolated nucleic acid molecule encoding a NFR polypeptide according to claim 1, wherein the NFR amino acid sequence is at least 60% identical to either of SEQ ID NO: 8, 15 or 25.
 - An isolated nucleic acid molecule encoding a NFR 1 polypeptide according to claim 2, comprising the amino acid sequence selected from the group consisting of SEQ ID No: 24, 25, 52 and 54.

- 7. An isolated nucleic acid molecule encoding a NFR 5 polypeptide according to claim 3, comprising an amino acid sequence selected from the group consisting of SEQ ID No: 8, 15, 32, 40 and 48.
- 8. An isolated nucleic acid molecule which encodes a NFR1 polypeptide having a specific Nod-factor binding property, and which hybridises with a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID No: 21, 22, 23, 51 and 53 under stringency conditions of no less than about 1.0xSSC at 65°C.

10

15

25

30

- 9. An isolated nucleic acid molecule which encodes a NFR5 polypeptide having a specific Nod-factor binding property, and which hybridises with a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID No: 6, 7, 11, 12, 39 and 47 under stringency conditions of no less than about 1.0xSSC at 65°C.
 - 10. An expression cassette comprising a nucleic acid molecule according to any one of claims 5 to 9.
- 20 11. The expression cassette of claim 10, wherein the nucleic acid molecule encoding a NFR polypeptide is operably linked to a transcriptional regulatory element.
 - 12. A vector comprising the expression cassette of claim 11.
 - 13. A cell that is stably transformed with the expression cassette of claim 11.
 - 14. The cell according to claim 13, wherein said cell is a plant cell.

WO 2005/003338

5

10

15

25

PCT/DK2004/000478

71

- 15.A method of producing a plant expressing a Nod-factor binding element, the method comprising introducing into the plant a transgenic expression cassette comprising a nucleic acid sequence encoding a NFR polypeptide according to any one of claims 5 to 9, wherein the nucleic acid sequence is operably linked to a promoter and selecting transgenic plants and their progeny expressing said NFR polypeptide.
 - 16. The method of claim 15, wherein the transgenic expression cassette is introduced into the plant through a sexual cross.
 - 17. The method of claim 15, wherein said promoter is a native or heterologous root specific promoter.
- 18. The method of claim 15, wherein said promoter is a native or heterologous constitutive promoter.
- 19. A transgenic plant expressing one or more NFR polypeptides produced according to the method of any one of claims 15 to 18.
- 20. The transgenic plant of claim 19, expressing the Nod-factor binding element according to any one of claims 1 to 4, and having a specific Nod-factor binding property.
 - 21. The transgenic plant of claim 19 or 20, wherein the plant is a non-nodulating dicotyledenous plant.
 - 22. The transgenic plant of claim 19 or 20, wherein the plant is a monocotyledonous cereal.
- 30 23. A method for marker assisted breeding of NFR alleles, encoding variant NFR polypeptides, comprising the steps of:

5

10

15

20

a. identifying in a nodulating legume species a variant NFR polypeptide having specific Nod-factor binding properties and having an amino acid sequence substantially similar to a sequence selected from the group consisting of SEQ ID No: 8, 15, 24, 25, 32, 40, 48, 52 and 54, and

 b. determining the nodulation frequency of legume plants expressing said variant NFR polypeptide, and

- identifying a DNA polymorphism at a locus genetically linked to or within the allele encoding said variant NFR polypeptide, and
- d. preparing a molecular marker based on said DNA polymorphism, and
- e. using said molecular marker for the identification and selection of a plant carrying an NFR allele encoding said variant NFR polypeptide.
- 24. A plant selected according the method of claim 23, carrying a *NFR* allele encoding a variant NFR polypeptide.
- 25. Use of the method of claim 23 for breeding a plant with enhanced nodulation frequency and/or root nodule occupancy and/or enhanced symbiotic nitrogen fixation ability.
- 26. A use according to claim 25, wherein said plant is a legume.